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Phytochemicals in Japanese plums: Impact of maturity and bioaccessibility

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Abstract

In recent years there has been increasing consumer interest in the potential health benefits of dietary derived phytochemicals such as polyphenols (including anthocyanins and flavonols) and carotenoids. A new variety of Japanese plum (*Prunus salicina* Lindl.), named Queen Garnet (QG), was developed as a high anthocyanin plum in a Queensland (Australia) Government breeding program and may be attractive to consumers, but knowledge of other phytochemical content, and bioaccessibility, is currently limited. As a result, the present study examined (1) the impact of harvest date on anthocyanins, quercetin glycosides and carotenoids in Queen Garnet and another red fleshed commercial Japanese plum variety, Black Diamond (BD), (2) the content of bound phenolics in plum fruit and (3) the *in vitro* bioaccessibility and release of these phytochemicals as an initial measure to predict their potential bioavailability. For both QG and BD, the last harvest resulted in the highest anthocyanin content in peel, flesh and whole fruit, whereas no significant effects could be observed for quercetin glycosides, and total carotenoids decreased over time. The highest content of bound phenolics (30% of total amount) could be found in BD flesh. Between 53% and 59% of quercetin glycosides and anthocyanins was released from QG after the gastric and small intestinal digestion procedure, whereas the release of carotenoids ranged between 4-6%. A relative high release of anthocyanins and quercetin glycosides could be observed from QG which may result in a higher gastro-intestinal absorption rate of these compounds. However, follow-up studies (clinical trials) are warranted to investigate the *in vivo* bioavailability and subsequently biological activity of QG.

Keywords: Japanese plums; phytochemicals; maturity; *in vitro* digestion; bioaccessibility

1. Introduction

The role of dietary phytochemicals, such as polyphenols and carotenoids, in human health and well-being is a major research topic in the last decade. Anthocyanins (e.g. cyanidin glycosides) and flavonols (e.g. quercetin glycosides), two main polyphenol subclasses, are some of the most abundant polyphenols in fruits and vegetables with an estimated daily intake of up to 64.9 mg anthocyanins and 54.9 mg flavonols, respectively (Zamora-Ros, Knaze, Lujan-Barroso, Slimani, Romieu, Fedirko, et al., 2011; Zamora-Ros, Knaze, Lujan-Barroso, Slimani, Romieu, Touillaud, et al., 2011). Recent publications indicate that the consumption of dietary anthocyanins as fresh food, juice, puree or powder may exert protection against cardiovascular risk factors, type 2 diabetes, oesophageal cancer and deterioration of bone tissues in humans (Cassidy et al., 2011; Chen et al., 2012; Hassellund et al., 2013; Jennings et al., 2012; Jennings, Welch, Spector, Macgregor, & Cassidy, 2014; Welch et al., 2012; Zhu et al., 2013).

Although afforded much less attention than some other phytochemical-rich fruits, certain varieties of Japanese plum (*Prunus salicina* Lindl.) are very significant sources of dietary anthocyanins (such as the variety Queen Garnet [QG]) and quercetin glycosides (K. Fanning, Topp, Russell, Stanley, & Netzel, 2014; Venter, Joubert, & de Beer, 2013). It has been previously shown in pilot trials that anthocyanin content increases with maturity in QG (K. Fanning et al., 2013) and other plums (K. Fanning et al., 2014) but content of quercetin glycosides and carotenoids are of interest but have not been studied. Furthermore the so-called bound phenolic fractions, including condensed tannins and hydrolysable polyphenols, which are generally not determined in fruits when the content is analysed by conventional extraction techniques have not been previously described in Japanese plum. A recent study with European plum (*Prunus domestica*) showed that >82% of the total antioxidant activity was due to the bound fractions (Kristl, Slekovec, Tojnko, & Unuk, 2011). It is anticipated that these “missing dietary polyphenols”, which often reach the colon and are then subject to microbial

degradation, may mediate significant health benefits (Saura-Calixto, 2012; Vizzotto, Cisneros-Zevallos, Byrne, Ramming, & Okie, 2007).

Polyphenols and carotenoids must be released from the fruit matrix and then absorbed through the gut wall in some form to exert their systemic effects in the body. *In vitro* models that mimic the gastric and small intestinal digestion process are a common approach to determine the release/bioaccessibility of nutrients and phytochemicals as an initial measure to predict their potential bioavailability. Bioaccessibility is defined as the fraction of a nutrient (or phytochemical) that is released from a food matrix and potentially available for intestinal absorption (Parada & Aguilera, 2007). To date, no bioaccessibility data of the major phytochemicals in Japanese plum are available. The relatively high anthocyanin content in the flesh of dark red fleshed Japanese plum varieties such as QG is a novel feature compared to other anthocyanin-rich fruits such as many berries and red grapes, where anthocyanins are almost exclusively in the peel. As a consequence, this may result in a higher *in vitro* bioaccessibility and subsequently *in vivo* bioavailability.

Therefore, the objectives of the present study were (1) to assess the impact of maturity on anthocyanins, quercetin glycosides and carotenoids in QG and another red fleshed Japanese plum variety, Black Diamond (BD), (2) to determine the content of nonextractable (bound) phenolic compounds in peel, flesh and whole fruit and (3) to evaluate the bioaccessibility and release of these phytochemicals as an initial measure to predict their potential bioavailability using an *in vitro* digestion procedure.

2. Material and methods

2.1. Chemicals

Unless otherwise stated, all chemicals were from Merck (Darmstadt, Germany), Scharlau Chemie S.A. (Barcelona, Spain) or Sigma-Aldrich (Sydney, NSW, Australia) and were of HPLC or analytical grade. Throughout the experiments, deionized water

was used (MILLIPORE Australia Pty Ltd, Kilsyth, VIC, Australia). Cyanidin-3-glucoside and cyanidin-3-rutinoside were purchased from ChromaDex (Irvine, CA, USA). Lutein, zeaxanthin and β -cryptoxanthin were purchased from Extrasynthese (Genay, France). Quercetin-3-rutinoside (rutin), quercetin-3-glucoside, quercetin-3-galactoside and β -carotene were purchased from Sigma-Aldrich.

2.2. Sample Preparation

Fruit were harvested from trees grown at the Applethorpe Research Facility (Applethorpe, Queensland, Australia [latitude: -28.6217, longitude: 151.9533, elevation: 876 m]) as described for Queen Garnet previously (Fanning et al., 2013). QG fruit were harvested on 17 January, 24 January, 31 January and 6 February (144, 151, 158 and 164 days post full bloom), and BD (cultivar Suplumeleven) fruit were harvested on 17 January, 24 January and 31 January (with no fruit left on trees on 6 February). On each date mature fruit (based on peel color) were picked and transported to Brisbane and stored at 1°C overnight. All plums, 12 for each harvest date of each variety, were cut manually into half with a stainless kitchen knife and the flesh and peel separated. The fresh weight of peel samples, once separated from flesh, was approximately 15% of total fruit weight. The samples were freeze-dried and cryomilled (Retsch MM301 mill, Haan, Germany) prior to extraction.

For the *in vitro* digestion procedure fresh plums were picked in January 2013 from Applethorpe Research Facility, transported to Brisbane, stored overnight at 1°C and processed the next day as detailed below.

2.3. Extraction

Polyphenols (anthocyanins and quercetin glycosides) were extracted as described previously (K. Fanning et al., 2013).

Carotenoids were extracted following the method published by Fanning et al. (2010) with the following modifications. Approximately 0.2 g was weighed into 50 mL tubes, 10 mL acetone (as the initial extraction solvent) was added and then the tubes were vortexed for 20 s. Samples were then handled as previously described, with hexane added to partition carotenoids into organic layer, centrifugation to separate the organic from aqueous layers, centrifugal evaporation to dry the organic fractions and reconstitution for HPLC analysis (K. J. Fanning et al., 2010).

The non-extractable or bound phenolics were extracted using a method that was adopted from Kristl *et al.* (2011). To the residue left from the polyphenol extraction, 10 mL of acetone was added and then vortexed and centrifuged at 5000 rpm for 10 min. The supernatant was then removed and the residue was stored over night at -84 °C and then freeze-dried to remove any residual solvent. Two mL methanol and 0.2 mL concentrated sulphuric acid were then added to dried residue (approximately 0.15 g) and incubated for 20 h in a shaking water bath (100 rev/min) at 85 °C. Following this the samples were centrifuged (10 min at 5000 rpm), and the supernatant (termed the 'hydrolysable tannins' fraction), was transferred to another 15 mL tube and diluted to 10 mL (with water). 0.5 mL acetone and 3 mL butanol/HCl (95/5, v/v) were then added to the residue, vortexed (1 min) and placed in a shaking water bath (100 rev/min) at 95 °C for 40 min. Following centrifugation (10 min at 5000 rpm), the supernatant (termed the 'proanthocyanidin' fraction), was transferred to another 15 mL tube and was diluted to 5 mL with butanol. These fractions were diluted with ethanol prior to analysis. All samples were then analyzed for total phenolic content (free and bound) by Folin-Ciocalteu reagent as detailed below.

2.4. *In vitro* digestion

Three large BD and three large QG fruits were separately cut into 12 pieces (without seed) and blended with a kitchen blender (Breville WIZZ stick, Breville, Sydney, NSW,

Australia) to chop the plums. *In vitro* digestion procedure, adopted from Netzel et al. (2011) with slight modifications, was undertaken using this plum material. Briefly, ~5 g of either BD or QG was weighed into 10 individual 50 mL tubes and the remaining BD and QG material was stored separately as reference starting material at -84 °C until analysis. Five independent digestion trials were carried out whereas five tubes (one per trial) were removed after gastric digestion and the other five (one per trial) after gastric and small intestinal digestion to measure the impact of the two digestion compartments on the bioaccessibility/release of the hydrophilic/lipophilic plum phytochemicals. For the digestion procedure, 6 M HCl was added, with thorough mixing, until the samples reached pH 2 and then 250 µL of pepsin solution (40 mg/mL pepsin from porcine gastric mucosa (P7000, Sigma-Aldrich) dissolved in 0.1 M HCl). The tubes were incubated for 1 h at 37 °C in a shaking water bath (85 rev/min). After 1 h the gastric samples were removed, centrifuged (10 min, 5000 rpm) and the supernatants were stored at -84 °C until analysis. 0.1 M NaHCO₃ with calcium (824 mg CaCl₂*2H₂O in 500 mL 0.1 M NaHCO₃) was added until the pH reached 5.7. The samples were mixed well and incubated for a further 30 min at 37 °C in a shaking water bath (85 rev/min). The pH was increased from 2 to 5.7 to simulate the slow increase of the pH between the gastric and small intestinal digestion. After removing the small intestinal samples from the water bath, 1 M NaOH was added until pH of 7.0 was reached, with thorough mixing throughout. One ml of pancreatin-bile solution (2 mg/ml pancreatin from porcine pancreas (P1750, Sigma-Aldrich), 12 mg/ml porcine bile extract (B8631, Sigma-Aldrich) in 0.1 M NaHCO₃) was then added and mixed well. . Samples were incubated for another 2 h at 37°C in a shaking water bath (85 rev/min). After removing from the water bath the samples were centrifuged (10 min, 5000 rpm) and supernatants were then transferred into 15 mL tubes. Samples which were designated for polyphenol analysis were mixed with 500 µL formic acid (90%). All samples were then stored at -

84°C until extraction and analysis of anthocyanins, quercetin glycosides and carotenoids.

2.5. Determination of total phenolics, anthocyanins, quercetin glycosides and carotenoids

Total phenolic content (Folin-Ciocalteu method) was determined as previously described (Netzel et al., 2012) and results were expressed as mg of gallic acid equivalents per 100g (mg GAE/100 g). Anthocyanins and carotenoids were both analyzed by HPLC as detailed previously (K. J. Fanning et al., 2010; Netzel et al., 2012). Quercetin glycosides were analyzed with the same HPLC system as outlined in Fanning et al. (2010). The samples were diluted in water and filtered prior to injection onto a Scientific AcclaimTM PolarAdvantageII C18 3 μ m 120 Å (4.6 × 150 mm) column (Thermo Scientific, Copacabana, NSW, Australia). The mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in acetonitrile. A gradient with a flow of 1.5ml/min was used: 0 min, 20% phase B; 12 min, 40% phase B; 21 min, 60% phase B; 23 min, 80% phase B; 27 min, 80% phase B; 29 min, 20% phase B; 35 min, 20% phase B. Identification and quantitation of quercetin/quercetin glycosides was undertaken at 355 nm using the following standards, quercetin-3-rutinoside (rutin), quercetin-3-glucoside, quercetin-3-galactoside, quercetin and comparison with literature (Jaiswal et al., 2013; Venter et al., 2013).

2.6. Statistics

The significance of differences between harvest dates or plum type were evaluated using one-way analysis of variance (ANOVA) and the Tukey HSD procedure using JMP software (Version 7; SAS, Cary, NY, USA). Differences were considered significant when p values were below 0.05.

3. Results

3.1. Anthocyanins and quercetin glycosides

The last harvest resulted in the highest anthocyanin content (sum of cyanidin-3-glucoside and cyanidin-3-rutinoside) in peel, flesh and whole fruit with QG (Fig 1A) having significantly higher ($p < 0.05$) levels than BD (Fig. 1B). Cyanidin-3-glucoside was the predominant anthocyanin in both varieties (Table 1). The relative anthocyanin content in QG flesh (% of total (whole fruit) content) was significantly higher ($p < 0.05$) than in BD at all harvest dates with an observed maximum of 47% on 6 February (Table 2).

Quercetin-3-glucoside and quercetin-3-rutinoside were identified as the main quercetin glycosides in QG and BD (Table 1). There was a significant ($p < 0.05$) increase in quercetin glycosides in peel of both plum cultivars, as well as the whole fruit of BD, between the first and the last harvest date (Fig. 2). Overall, QG had significantly ($p < 0.05$) higher levels in peel, flesh and whole fruit than BD. On 31 January (last harvest date of BD), 24% and 27% of quercetin derivatives were located in the flesh of QG and BD, respectively. However, an increase to 41% could be observed in QG flesh on 6 February (Table 2).

3.2. Free and bound phenolics

The average concentrations of free and bound phenolics in peel, flesh and whole fruit ranged between 165.3 and 965.5 mg GAE/100 g for free phenolics, between 14.0 and 27.1 mg GAE/100 g for hydrolysable tannins and between 52.4 and 73.4 mg GAE/100 g for proanthocyanidins. For both plum cultivars, the relative content (% of total (free and bound) content) of bound phenolics was higher ($p < 0.05$) in the flesh than in the peel, with BD having the highest content of 30 % (Fig. 3).

3.3. Carotenoid content

Five carotenoids could be quantified in QG, namely lutein, β -cryptoxanthin, β -carotene, zeaxanthin and α -carotene (the latter was found only in the peel) (Table 1). The slight decrease in total carotenoids in the peel, between the first and last harvest date, was not significant, whereas it was significant ($p < 0.05$) in the flesh and whole fruit (Fig. 4A). Lutein, β -cryptoxanthin and β -carotene could be quantified in BD (Table 1). There was a significant decrease ($p < 0.05$) in total carotenoids in the peel between the first and the last harvest date, whereas the slight decrease in the flesh and whole fruit was not significant (Fig. 4B).

3.4. *In vitro* digestion

The anthocyanin release after the mimicked gastric and small intestinal digestion process was similar in both plum cultivars with 56% for BD and 59% for QG, respectively (Table 3). However, the release of quercetin glycosides was significantly ($p < 0.05$) higher for QG with 53% released in total compared to 45% from BD (Table 2). The amount of released carotenoids was relatively low (and similar) in both plum cultivars with 4% and 6% for QG and BD, respectively (Table 3).

4. Discussion

4.1. *Anthocyanins and quercetin glycosides*

The anthocyanin content has been seen to increase in whole fruit, flesh and peel of Japanese plums with increasing maturity on tree (Diaz-Mula et al., 2008; K. Fanning et al., 2013; Netzel et al., 2012). Literature values for flesh and peel of commercial varieties have ranged from 0.5-17.7 mg/100 g and 12.9-916 mg/100 g (Diaz-Mula et al., 2008; Netzel et al., 2012; Tomas-Barberan et al., 2001). Whereas the peel content of QG and BD was in the range of these reported levels, the flesh content of QG and to a lesser extent BD, was outstanding being 6 and 1.6 times greater than the top of this range. The flesh levels of QG, at the most mature stage, were ~20% higher than other

high anthocyanin selections (85-87 mg/100 g, Cevallos-Casals, Byrne, Okie, and Cisneros-Zevallos (2006)). Whole fruit anthocyanin content for red-dark peeled and red fleshed commercial varieties often range from 10-80 mg/100 g (Chun, Kim, Moon, Kang, & Lee, 2003; Diaz-Mula et al., 2008; Netzel et al., 2012). QG and BD had levels above this range, with QG content (K. Fanning et al., 2013) comparable to or higher than breeding selections with dark peel and red-dark flesh (Cevallos-Casals et al., 2006). Dark red fleshed plums are a relatively unusual matrix given the fact that many anthocyanin-rich fruits have these pigments heavily concentrated in the peel/skin, including grapes (> 99% in skin, Rebello et al. (2013)) and many berries. QG had an average of 45% of anthocyanin content of the whole fruit in the flesh and BD had 28%. Whilst many commercial varieties of Japanese plum also have only a small fraction of total anthocyanin content in flesh (K. Fanning et al., 2014), high anthocyanin selections in other breeding programs have also shown high flesh contributions (up to 71%) to total anthocyanin content (Cevallos-Casals et al., 2006).

There is no literature data that we are aware of quercetin/quercetin glycosides in whole fruit during ripening (Fanning et al., 2014). Tomas-Barberan et al. (2001) showed that peel flavonol glycosides both increased and decreased, depending on cultivar on ripening. The content of quercetin glycosides in the peel of selected cultivars has been shown to range from 16.6 to 35.2 mg/100 g (Black Beaut) (Tomas-Barberan et al., 2001). Both plums had higher peel content, being 2.3 (BD) and 5.4 (QG) times higher than Black Beaut. Mubarak et al. (2012) analyzed 29 prevarietal selections and saw flavonol glycoside content of 0.9-27.9 mg/100 g fruit. Other commercial varieties have had levels of 2.3-27.0 mg/100 g fresh weight (Chun et al., 2003; Harnly et al., 2006; Ozturk, Kucuker, Karaman, & Ozkan, 2012; Proleggente et al., 2002; Venter et al., 2013) and the content of BD was in this range. However, the levels in QG were higher being 2-fold that of the highest literature values for commercial Japanese plum varieties

and comparable or greater than levels seen for significant dietary sources of quercetin glycosides including onions (Proteggente et al., 2002).

4.2. Bound phenolics

Standard extraction methods have been appraised as underestimating the actual total amount of phenolic compounds in certain food products (Saura-Calixto, 2012). Saura-Calixto (2012) reported, that the “extractable [free] polyphenols may be only the tip of the iceberg”. The work of Kristl *et al.* (2011) in four European plum cultivars showed that the free antioxidants (extracted using acidified methanol followed by acetone:water) accounted for less than 18% of the total antioxidant activity. However it is important to note that fresh, not freeze-dried plums were used for extraction in that study. Several possible reasons for the relatively low % (~20%) of bound phenolics, in QG and BD, include the high content of anthocyanins relative to the European plum cultivars, which are often nearly completely extractable in acidified methanol following homogenization (Yousef et al., 2013), and the fact that QG/BD samples were freeze dried prior to extraction. The use of freeze-drying has been shown to significantly enhance the extractability of several phenolic acids in blueberries (Yousef et al., 2013). However, even this relatively low content of bound phenolics in QG and BD may be of physiological significance. A meal of two large QG plums (~200 g) would easily provide up to 140 mg bound phenolics according to our present findings. It is suggested that these plant matrix/plant cell wall bound phenolics are transported to the colon without further degradation and/or absorption (Saura-Calixto 2012). Reaching the colon, these compounds may either be fermented by the colonic microbiota forming absorbable metabolites such as phenylacetic and phenylbutyric acids or could contribute to the maintenance of a healthy gut environment via prebiotic-like effects (Cardona, Andres-Lacueva, Tulipani, Tinahones, & Queipo-Ortuno, 2013; Padayachee et al., 2013). However, these compounds may also remain bound to the plant matrix and

therefore be eliminated in faecal waste without any further interactions with the colonic microbial population (Padayachee et al., 2013).

4.3. Carotenoid content

The carotenoid content of flesh, peel and whole fruit of QG and BD were in the range of previous values for commercial Japanese plum varieties, flesh: 0.056-1.1 µg/g, peel: 0.27-9.9 µg/g, whole fruit: 0.09-1.9 µg/g (Diaz-Mula et al., 2008; Gil, Tomas-Barberan, Hess-Pierce, & Kader, 2002). In contrast to a decrease in carotenoid content with harvest date, in the present study, a previous study showed increases in both peel and flesh with longer time on tree in other varieties (Diaz-Mula et al., 2008). Varietal differences are most likely the reason for this observation and have been evidenced for changes in carotenoid content during postharvest storage (Diaz-Mula et al., 2011).

4.4. *In vitro* digestion

The release of anthocyanins from QG and BD after gastric and small intestinal digestion was relatively high, with QG having a 3% higher release than BD (59 vs. 56%, $p > 0.05$). These findings are in line with results of *in vitro* release of anthocyanins reported by others. For example, Bermudez-Soto et al. (2007) recovered ~57% of cyanidin-3-glucoside and ~58% of total anthocyanins in the digesta after *in vitro* pancreatic digestion of chokeberry juice. However, “*in vitro* recoveries” as low as 20% for pomegranate anthocyanins were also reported in the literature (Perez-Vincente et al. 2002). The chemical structure of dietary anthocyanins, the food/fruit matrix and subsequently the exposure to the alkaline conditions of the pancreatic digestion process are regarded as the main reasons for these inconsistent results (Bermudez-Soto et al. 2007). The food matrix can play a particularly significant role in the stability of polyphenols/anthocyanins with reports that polyphenols transiently bind to food matrices (e.g. plant cell wall components) during the gastro-intestinal digestion, which

may protect them (and especially the more labile anthocyanins) from degradation processes (Padayachee et al. 2013; Parada and Aguilera 2007). The observed higher release of anthocyanins (trend, $p > 0.05$) from whole QG fruit compared to BD as a potential result of the higher flesh:total anthocyanin ratio needs to be investigated in a follow-up study. Finally, it should be also noted that the relative amount of anthocyanins released after gastric digestion was in the same range as reported for anthocyanins from purple figs, with up to 35% cyanidin-3-rutinoside in the gastric digesta (Kamiloglu and Capanoglu 2013).

The relative release (released amount vs. total amount) of quercetin-glycosides from QG and BD fruit after *in vitro* digestion was lower than it was for the anthocyanins. However, the difference between QG and BD (53% vs. 45% of released quercetin glycosides) was significant ($p < 0.05$) and may be due to the higher flesh:total quercetin glycoside ratio as already suggested for the anthocyanins. The observed amount of bioaccessible quercetin-glycoside in the present study is comparable with the results reported by Bermudez-Soto et al. (2007): recoveries of ~81%, ~70% and ~73% for quercetin-3-glucoside, quercetin-3-rutinoside and total flavonols, respectively, from chokeberry juice were found at the end of the *in vitro* digestion process. Boyer et al. (2005) reported a recovery of almost 90% of quercetin-3-glucoside after the *in vitro* digestion of onions. Kamiloglu and Capanoglu (2013) also saw a significant difference in the bioaccessible fraction of quercetin-3-rutinoside after gastric digestion of whole-fresh yellow and purple figs, ranging from 40% (yellow) to 107% (purple). The food/fruit matrix and potential interactions with matrix components and compounds influencing the stability and/or accessibility of quercetin glycosides (and also anthocyanins) during the *in vitro* digestion process are most likely responsible for the observed differences.

A relatively low release of lipophilic carotenoids from the plum matrix was expected since the sample material was not thermally treated and oil or lipids were not added

(thermal (pre)treatment and the addition of oil/lipids to fruits and vegetables can significantly increase the released fraction of carotenoids (Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, 2009; Netzel et al., 2011; Parada & Aguilera, 2007)). Plums are commonly consumed raw and these findings are in line with the results reported by others (Hedren, Diaz, & Svanberg, 2002; Lemmens et al., 2009; Veda, Kamath, Platel, Begum, & Srinivasan, 2006): the relative release of β -carotene (which is also the main carotene in QG and BD) from raw carrots, amaranth and fenugreek leaves ranged between 3% and 11%.

However, several drawbacks and limitations of these *in vitro* digestion methods should be critically considered when interpreting the results: e.g. to date, no *in vitro* model is capable of covering all aspects of *in vivo* digestion and absorption, distribution, metabolism (including the metabolic activity of the gut microbiota) and elimination. Polyphenol metabolites generated *in vivo* are of particular interest in terms of their potential biological significance in the prevention of chronic diseases as well as the maintenance of a “healthy gut” (Tomas-Barberan and Andres-Lacueva, 2012; Williamson and Clifford, 2010). Therefore, human studies/clinical trials are still the “Gold Standard” to assess the bioavailability and metabolism (and subsequently bioactivity) of dietary polyphenols and carotenoids and yet cannot be replaced by any *in vitro* models.

5. Conclusions

QG has outstanding anthocyanin content and should be harvested as late as possible to maximise anthocyanins. The relative low content of bound phenolics in both plum varieties needs further investigation to elucidate if this is a plum/cultivar specific feature or due to other reasons such as the handling procedure of the sample material prior to the extraction process. A relative high release of anthocyanins and quercetin glycosides could be observed from QG which may result in a higher gastro-intestinal absorption

rate of these compounds. However, follow-up studies (clinical trials) are warranted to investigate the actual *in vivo* bioavailability and subsequently biological activity of QG.

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Tables

Table 1

Phytochemical composition in whole fruit of BD and QG at the last harvest date.

Plum cultivar/		BD	QG
Harvest date		31 Jan	6 Feb
Phytochemicals			
Anthocyanins	Total	89.8 ± 18 mg/100 g	180.7 ± 23 mg/100 g
		(100%)	(100%)
	Cya-3-gluc	73%	74%
	Cya-3-rut	27%	26%
Quercetin ^a /Quercetin glycosides	Total	17.3 ± 4.5 mg/100 g	56.1 ± 8.3 mg/100 g
		(100%)	(100%)
	Q-3-gluc and Q-3-rut	90%	83%
	Q-3-gal	10%	17%
Carotenoids	Total	0.8 ± 0.3 µg/g	1.0 ± 0.2 µg/g
		(100%)	(100%)
	Lutein	1%	7%
	β-cryptoxanthin	24%	11%
	Zeaxanthin	ND	9%
	α- and β-carotene	75% ^b	73%

Total: sum of detectable phytochemicals, means \pm standard deviation, n=12 fruits; Cya, cyanidin; gluc, glucoside; rut, rutinoside; gal, galactoside; Q, quercetin; ^anot in all samples and <1%; ^bonly β -carotene; ND, non-detectable.

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Table 2

Relative^a phytochemical content in flesh of BD and QG at different harvest dates.

Plum cultivar	BD			QG			
Harvest date	17 Jan	24 Jan	31 Jan	17 Jan	24 Jan	31 Jan	6 Feb
Phytochemicals	% in flesh						
Anthocyanins	22 ± 1	33 ± 8	29 ± 3	43 ± 4*	46 ± 6*	42 ± 3*	47 ± 4*
Quercetin/Quercetin glycosides	19 ± 5	20 ± 3	27 ± 7	29 ± 5*	30 ± 5*	24 ± 2	41 ± 4*
Carotenoids	61 ± 6	57 ± 6	75 ± 16	73 ± 14	55 ± 12	57 ± 12	57 ± 13

^acontent in the flesh as % of total (whole fruit) content; values are means ± standard deviation, n=12 fruits; Anthocyanins: sum of cyanidin-3-glucoside and cyanidin-3-rutinoside; Quercetin/Quercetin glycosides: sum of quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-galactoside and quercetin; Carotenoids: sum of lutein, β-cryptoxanthin, zeaxanthin, α- and β-carotene for QG and lutein, β-cryptoxanthin and β-carotene for BD; *p < 0.05, QG vs. BD.

Table 3

Release (bioaccessibility) of phytochemicals from QG and BD

Plum cultivar	Phytochemical content in plum puree		Released phytochemicals	
			Gastric digestion (60 min)	Gastric and small intestinal digestion (180 min)
Queen Garnet	Anthocyanins (mg/5 g):	10.0 ± 0.08	3.4 ± 0.39 (34%) ^a	5.8 ± 0.40 (59%)
	Quercetin/Quercetin glycosides	3.2 ± 0.03	0.6 ± 0.08 (20%)	1.7 ± 0.15 (53%)*
	(mg/5g):	19.2 ± 0.24	ND	0.8 ± 0.08 (4%)
	Carotenoids (µg/5 g):			
Black Diamond	Anthocyanins (mg/5 g):	4.5 ± 0.07	1.7 ± 0.09 (38%)	2.5 ± 0.19 (56%)
	Quercetin/Quercetin glycosides	1.2 ± 0.02	0.2 ± 0.01 (16%)	0.5 ± 0.05 (45%)
	(mg/5 g):	15.6 ± 0.26	0.5 ± 0.05 (3%)	0.9 ± 0.09 (6%)
	Carotenoids (µg/5 g):			

values are means ± standard deviation, n=5 independent trials; Anthocyanins: sum of cyanidin-3-glucoside and cyanidin-3-rutinoside; Quercetin/Quercetin glycosides: sum of quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-galactoside and quercetin; Carotenoids: sum of lutein, β-cryptoxanthin,

zeaxanthin, α - and β -carotene for QG and lutein, β -cryptoxanthin and β -carotene for BD; ^arelative release of phytochemicals (released amount vs. applied dose); %-data are rounded; * $p < 0.05$, QG vs. BD; ND, non-detectable.

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Figures

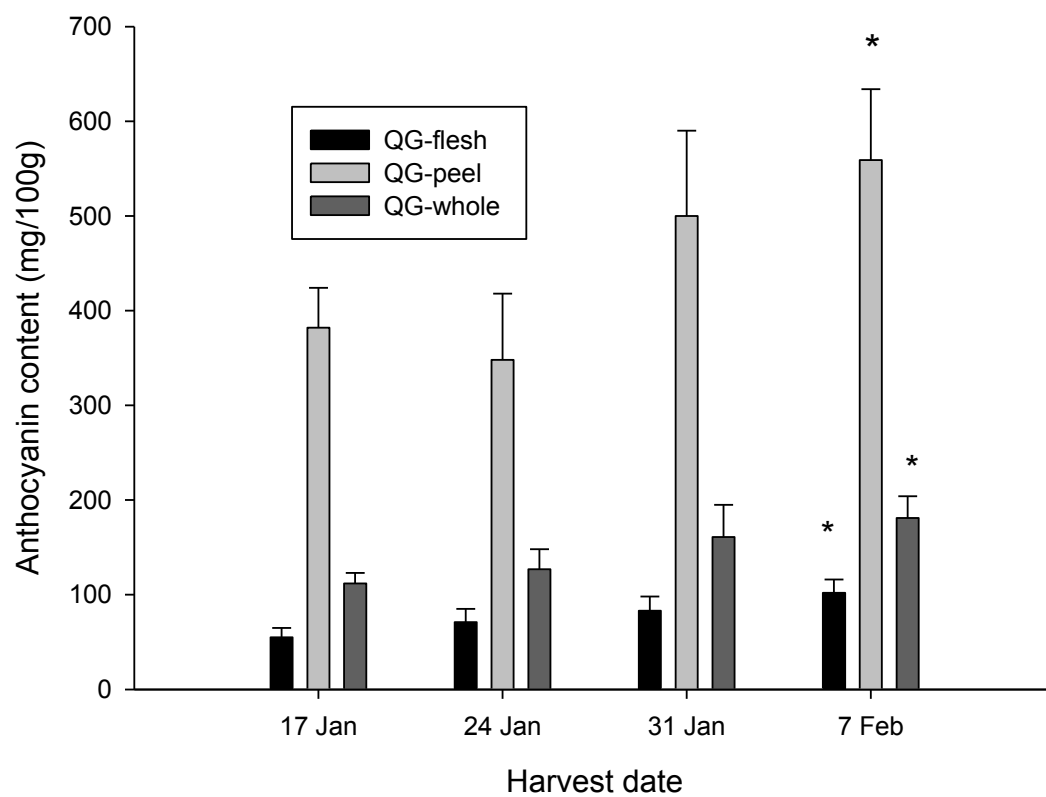
Fig. 1. Anthocyanin content in QG (A) and BD (B) across harvest dates (means \pm standard deviation, n=12; *p < 0.05 to first harvest date).

Fig. 2. Total quercetin glycoside content in QG (A) and BD (B) across harvest dates (means \pm standard deviation, n=12; *p < 0.05 to first harvest date).

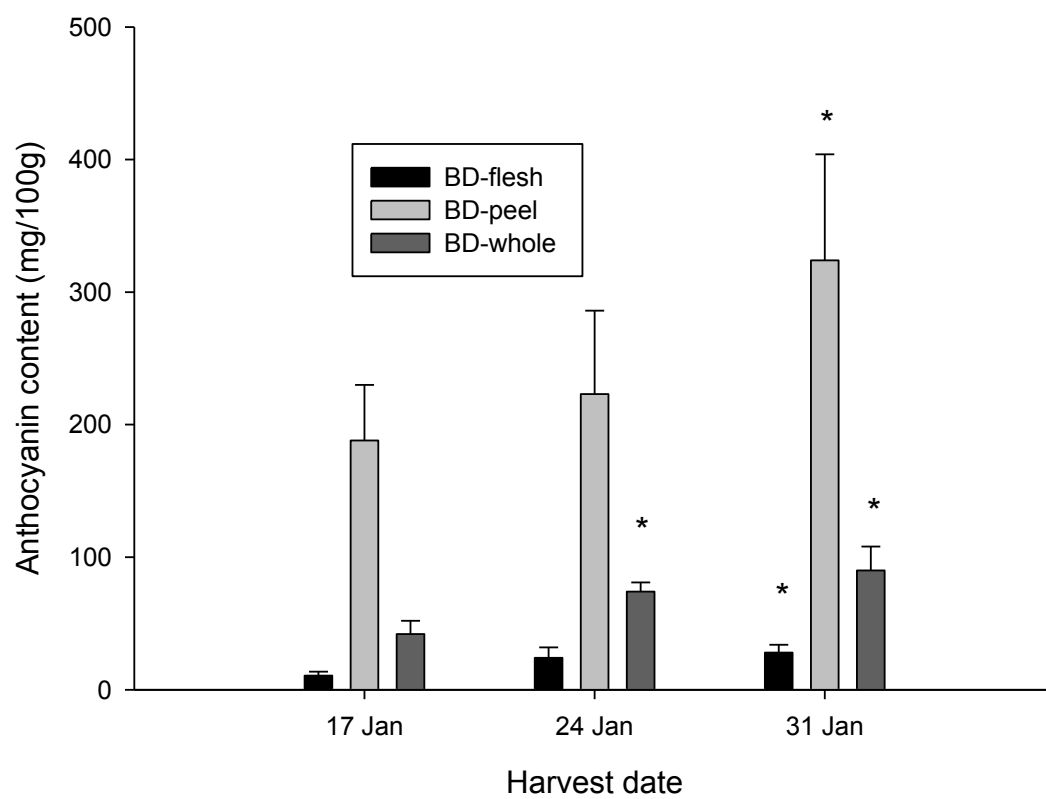
Fig. 3. Ratio of free to bound (hydrolysable tannins and proanthocyanidins) total phenolic content in QG (A) and BD (B) (means, n=12).

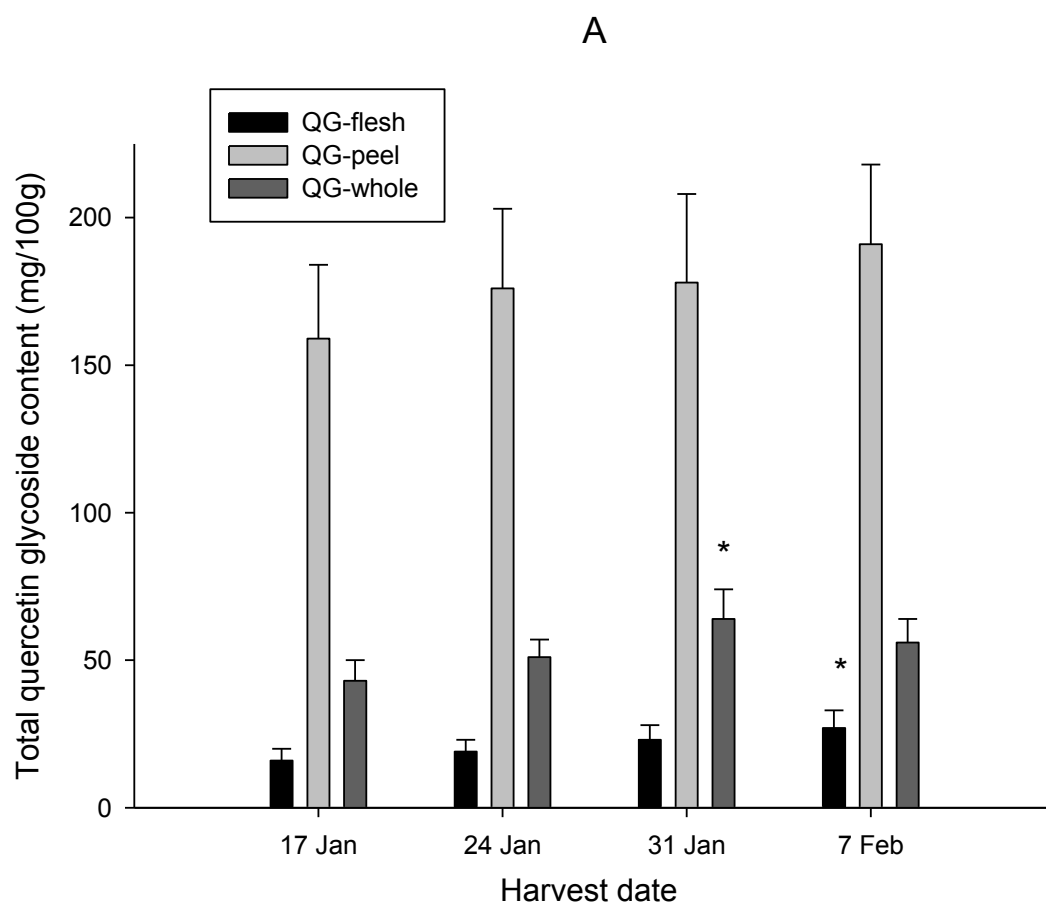
Fig. 4. Total carotenoid content in QG (A) and BD (B) across harvest dates (means \pm standard deviation, n=12; *p < 0.05 to first harvest date).

A



B

**Figure 1**



B

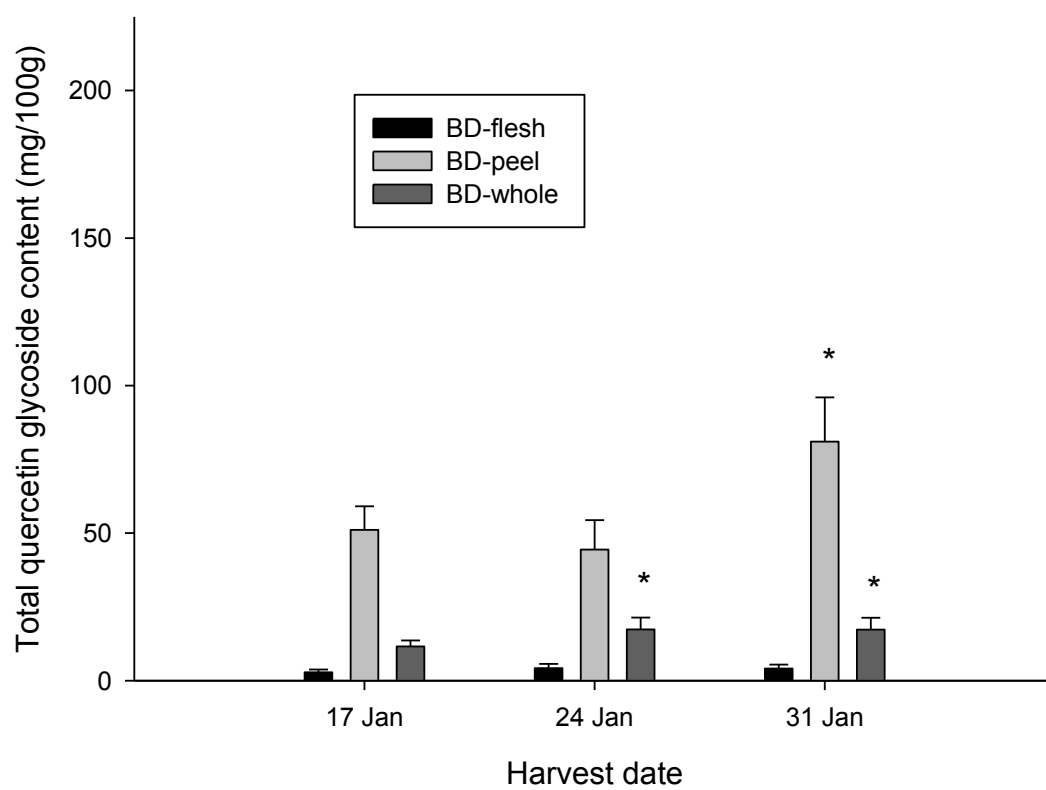


Figure 2

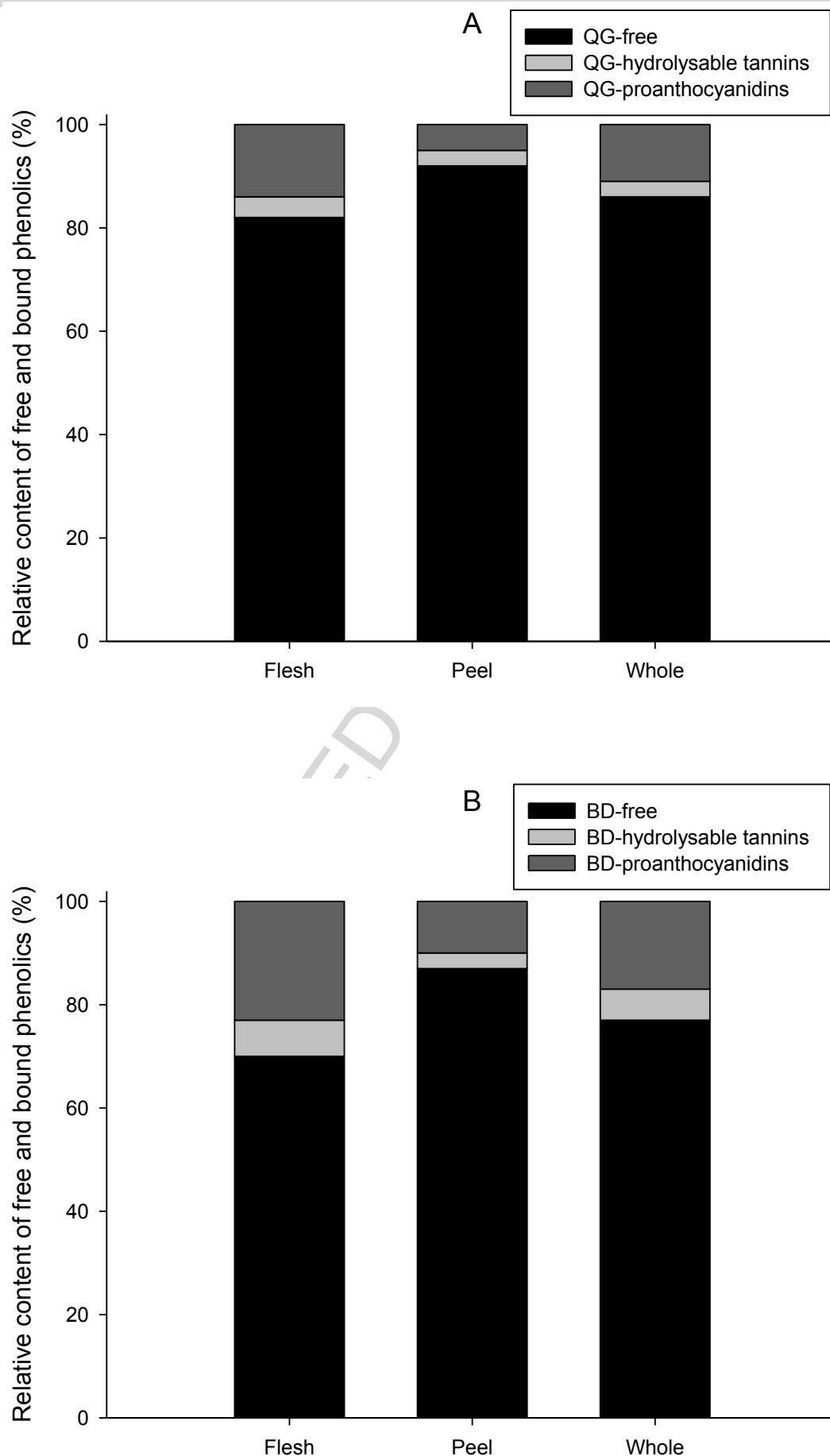
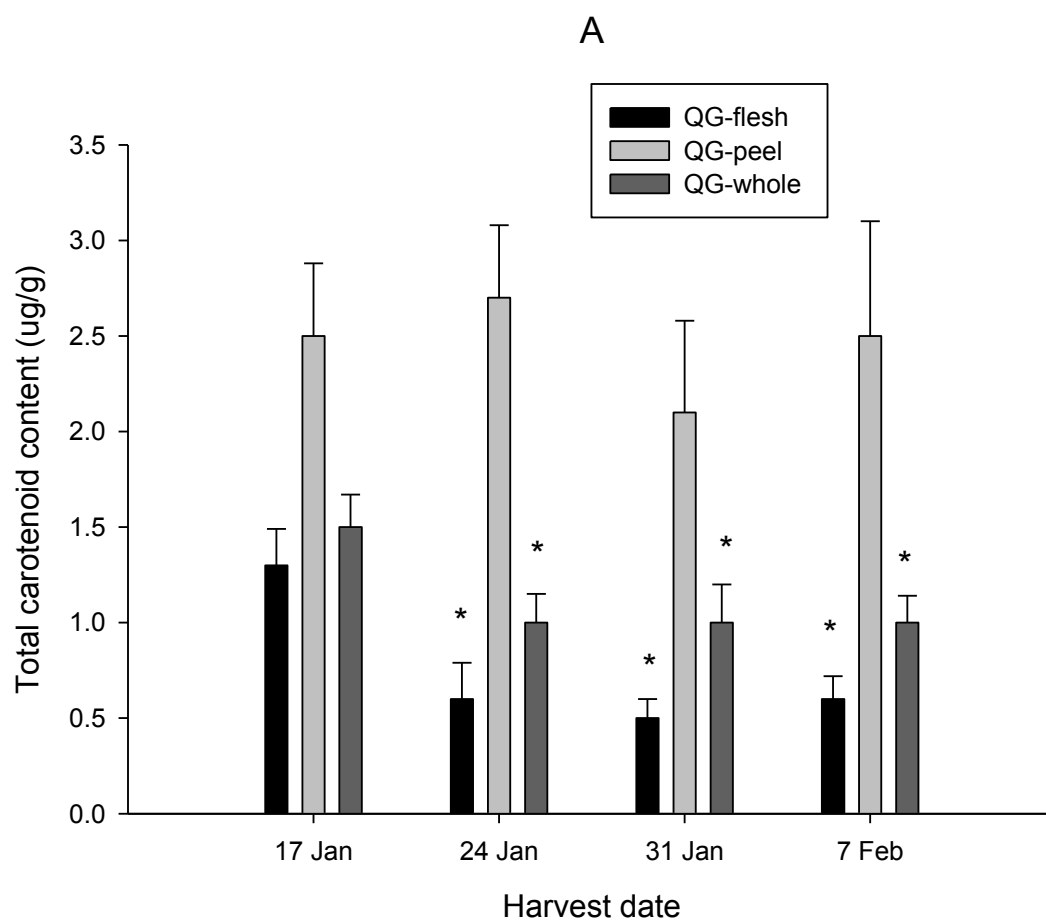


Figure 3



B

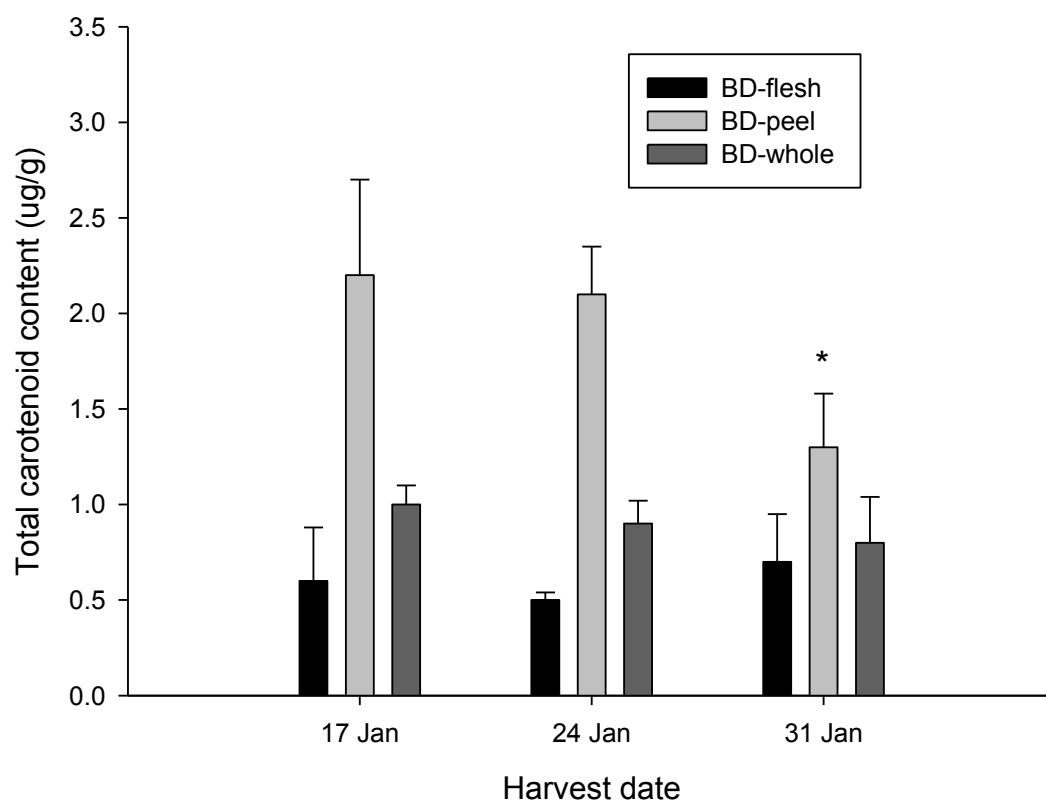


Figure 4

Highlights

- Queen Garnet (QG) has outstanding anthocyanin content.
- Later harvest maximises anthocyanins in QG and Black Diamond.
- Relatively low content of bound phenolics in both plum cultivars.
- Relatively high release of anthocyanins and quercetin glycosides from QG.